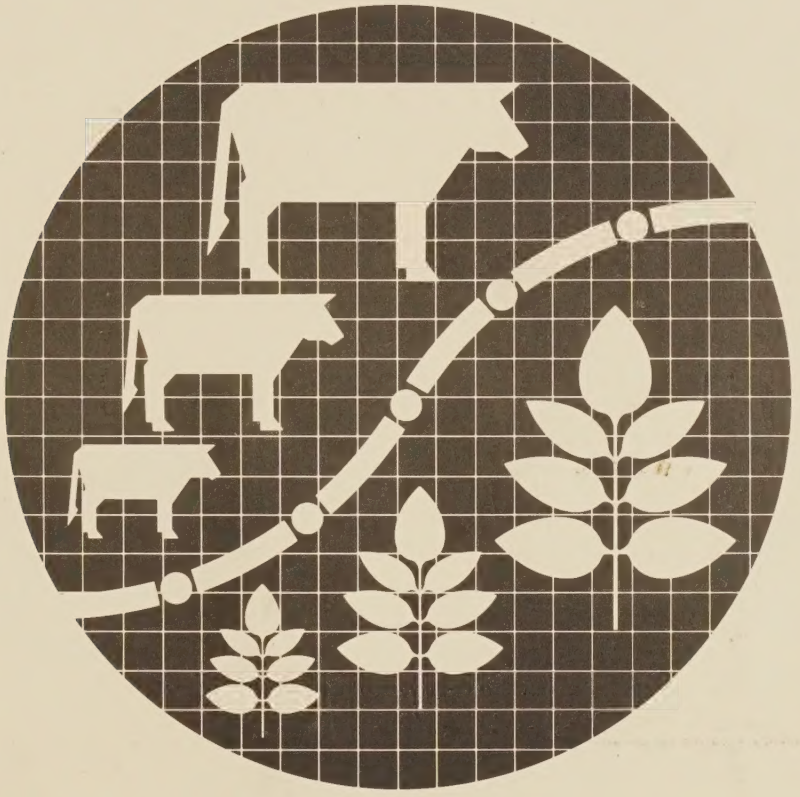
Beltsville Symposia in Agricultural Research



XII. Biomechanisms Regulating Growth and Development: Keys to Progress

May 3-7, 1987

ABSTRACTS

POSTER SESSION
May 6, 1987
Building 003
10:30 AM — 1:00 PM

United States Department of Agriculture

Agriculture Research Service
with the cooperation of
Friends of Agricultural Research
Beltsville, Inc.

Beltsville Symposia in
Agricultural Research

XII. Biomechanisms Regulating Growth and Development: Keys to Progress

Agricultural Research Service
United States Department of Agriculture

SECTION I

Abstracts Presented as Posters

Wednesday, May 6, 1987

10:30 am - 1:00 pm

1
DEVELOPMENTAL EFFECTS OF LONG-TERM SHELL-LESS CULTURE OF TURKEY EMBRYOS ON GROWTH AND TRACE ELEMENT METABOLISM

Richards, M.P.*, J.P. McMurtry, R.W. Rosebrough & N.C. Steele, USDA, Agricultural Research Service, Beltsville, MD 20705 USA

Turkey embryos were incubated in ovo or in shell-less culture (ex ovo) to 15, 18, 21 or 24 d of age. The ex ovo embryos grew at a slower rate and by d 24 were 44% smaller than those in ovo. Relative to body wt, liver and heart were larger in ex ovo embryos by d 24. Hematocrits were elevated in ex ovo embryos, perhaps due to limited growth of the chorioallantoic membrane in culture. Serum protein and Ca levels were lower in ex ovo embryos. In both groups, serum Zn levels declined, while Cu and Fe levels increased to 24 d of age. From d 15 to 24 in ovo liver Cu levels increased 4-fold while Zn and Fe declined, whereas, Zn and Fe levels increased while Cu did not in the ex ovo embryos. The ex ovo embryos exhibited a diabetic-like condition characterized by hyperglycemia, hypoinsulinemia, an inability of exogeneous insulin to suppress serum glucose and elevated pancreatic insulin which was unresponsive to the insulin secretagogue, arginine. By d 24 liver lipid content was reduced in ex ovo embryos, whereas serum lipid was increased, indicating impaired transfer of lipid to hepatic stores. We conclude that the ex ovo embryos exhibited a progressive Cu deficiency which may be related to the abnormal metabolism of Zn, Fe, glucose and lipid. Long-term shell-less embryo culture affords unique opportunities to study the role of trace elements in avian embryonic growth and development.

2
MEASUREMENT OF GLUCOSE AND LIPID METABOLISM IN AVIAN LIVER EXPLANTS.

ROSEBROUGH, R.W.* AND STEELE, N.C., USDA, Agricultural Research Service, Beltsville, MD 20705 USA

We used a MacIlwain^{R} tissue chopper and obtained liver slices from 21 to 28-d old chicks to determine assay conditions (substrates, buffers, time), regulators (metals and hormones) and points of endogenous regulation of de novo lipogenesis (ATPase, reductive potential and protein phosphorylation). High- and low-bicarbonate-based buffers (Earl's balance salts, EBSS and Hanks' balanced salts, HBSS; respectively) were used in conjunction with sources and types of bovine serum albumin (BSA), divalent cations (Mg^{2+} or Ca^{2+}), substrate (glucose or acetate) and hormones (insulin and catecholamines).

Neither a high- (EBSS) nor a low-bicarbonate (HBSS) buffer changed in vitro lipogenesis, CO_2 or glucose production. Likewise, neither the presence nor the source of bovine serum albumin (Sigma, Armour or Miles) affected metabolism. In contrast, changing the reaction surface area (5.1 vs 10.5 cm^2) decreased metabolic rates. Acetate was more readily utilized than glucose as an in vitro fatty acid precursor. Metals (Mg^{2+} or Ca^{2+}) had little affect upon lipogenesis. Insulin (porcine or chicken) did not change lipogenesis; however, incorporation of acetate into fatty acids was decreased by dibutyryl cyclic AMP. The slight amplification of this effect by theophylline indicates that the major point of lipid synthesis may be regulated by the cyclic AMP cascade.

3
CARCASS COMPOSITION AND PROTEIN METABOLISM IN PIGS SELECTED FOR LEAN GROWTH WHEN FED HIGH OR LOW PROTEIN DIETS.

Mitchell, A.D.*, Bereskin, B., Steele, N.C. and Solomon, M.B. USDA, Agricultural Research Service, Beltsville, MD 20705 USA

Barrows from the fifth generation of lines of pigs selected for lean growth when fed 12 (L) or 24% (H) protein diets were evaluated at different stages of growth for rate and efficiency of growth, carcass composition and protein utilization. In one experiment, select lines (HS and LS) were compared with control lines (HC and LC) at 30, 60 and 90 kg, with each line having been fed its designated diet starting at 8 wk of age. In contrast to previous results with the fourth generation, differences between C and S lines were only marginal. The HS line had less carcass lipid ($p < .05$) than HC, however LC grew more rapidly than LS and there were no differences between C and S lines with respect to muscle protein deposition and feed efficiency. In a second experiment barrows of the LS and HS lines of the fifth generation (second parity) were contrasted at 60 and 90 kg by dietary crossover in which pigs from each line were placed on either of the diets starting at 8 wk of age. On both diets the HS line deposited less lipid ($p < .05$) in the carcass and on the L diet more protein in carcass muscle. There were no differences in feed efficiency, however LS grew faster than HS on the L diet. In both studies determination of the efficiency of utilization of dietary protein for muscle growth and 5 day nitrogen balance trials at 60 kg (60 kg group only) indicated no differences in protein metabolism as a result of the selection program.

4
CELLULAR METABOLISM AND CYTOTOXICITY OF SUPPLEMENTAL IRON IN THE BABY PIG LIVER.

Caperna, T.J.*, Failla, M.L. and Steele, N.C., USDA, Agricultural Research Service, Beltsville, MD 20705 USA

Current management practices in the swine industry necessitate the administration of supplemental iron to newborns to prevent anemia. We have isolated and cultured hepatocytes, Kupffer cells and endothelial cells from livers of control and iron-treated piglets to investigate the metabolism and possible cytotoxic consequences of supplemental iron that is accumulated by each of these cell types. Kupffer cells and endothelial cells accumulated more iron than hepatocytes after the injection of iron-dextran. Monolayer cultures of Kupffer cells and endothelial cells also accumulated more ^{55}Fe-dextran than hepatocytes. Ferritin, the intracellular iron storage protein, was identified and quantitated in all three cell types by immunological methods. The concentration of ferritin-protein and the amount of iron associated with ferritin was greater in Kupffer and endothelial cells than in hepatocytes. Since high levels of cellular iron can be cytotoxic, lipid peroxidation was assessed by determining the levels of conjugated dienes in lipids extracted from intact livers and liver cells that were isolated from control and iron-dextran treated pigs. The level of conjugated dienes was higher in livers from iron-treated piglets than from controls. Hepatocytes contained higher concentrations of conjugated dienes than Kupffer cells or endothelial cells, indicating that the extent of lipid peroxidation is not necessarily correlated with intracellular iron content.

5
STUDIES ON MINERAL STATUS IN GROWING PIGS: EFFECTS OF GROWTH HORMONE TREATMENT AND ENERGY INTAKE.

Caperna, T.J.*, Campbell, R.G. and Steele, N.C., USDA, Agricultural Research Service, Beltsville, MD 20705 USA

Mineral status was investigated in a 2x3 factorial treatment arrray in a total of 36 barrows growing from 25-55 kg BWt. Treatments included three levels of feed intake (ad libitum, 1.64 and 1.35 kg feed/d) and exogenous growth hormone therapy (GH, 100 µg·kg $BWt^{-1}\cdot d^{-1}$ vs buffer injected controls). Blood was collected by venipuncture prior to sacrifice for the determination of trace element concentrations and hematocrit values. Concentration of iron in serum was markedly reduced in GH treated pigs subjected to moderate and severe feed restriction. In contrast, concentrations of copper and zinc in serum were similar in all groups. Hematocrit values were significantly lower in GH treated pigs compared to controls at all three levels of feed intake. The concentration of hepatic iron was lower in GH treated pigs and was reduced by 25% in GH treated pigs on severe feed restriction. However, since GH treatment resulted in greater liver mass, total hepatic iron was similar in GH treated and control pigs. Feed restriction resulted in a doubling of the concentration of hepatic iron in GH treated and control pigs. Hepatic zinc was unaffected by GH treatment and energy intake. These preliminary results indicate that energy intake and GH therapy can influence the metabolism of iron in growing swine and the respective treatments exert effects independently of one another.

6
EFFECT OF DIETARY ENERGY INTAKE (EI) AND GROWTH HORMONE (GH) THERAPY ON SERUM GH CONCENTRATION OF GROWING SWINE.

Steele, N.C.*, McMurtry, J.P., Brocht, D.M., Campbell, R.G., Caperna, T.J. and Rosebrough, R.W. USDA, Agricultural Research Service, Beltsville, MD 20705 USA

In a 2X2 factorial treatment array (n = 4/trt) the effects of EI (ad libitum vs 4.8 Mcal daily) and GH therapy (0, buffer injected, vs 100 µg porcine pituitary GH/kg BWt for the final 10 days of the trial) were assessed in barrows growing from 25 to 55 kg live weight. The circadian pattern of GH secretion was monitored as well as the pituitary response to porcine growth hormone releasing factor (GRF). All pigs were fitted with indwelling vena cava catheters at 55 kg and blood collected for serum harvest at 15 min intervals for 2 h prior and 10 h following GH injection. EI (0 GH) had no effect on basal serum GH nor frequency or amplitude of secretory pulses. Pigs treated with GH had similar basal GH concentrations (<1 ng/ml). Following GH treatment, concentrations peaked (26-53 ng/ml) between 60-150 min post-injection. Restriction of EI elevated integrated serum GH area 25% compared to pigs not restricted. The following day, pigs were sampled at 15 min intervals for 2.5 h prior to and at more frequent intervals for 3 h following GRF infusion (10 µg/kg BWt). GRF stimulated GH secretion within 20 min of infusion and serum GH remained elevated for 60 min (0-GH). Prior treatment with GH (100 GH) negated GRF-induced GH secretion.

7

SOMATOTROPIN'S EFFECT ON METABOLIC RESPONSE TO THE EUGLYCEMIC CLAMP IN GROWING SWINE

Wray-Cahen*, C. Diane, R. Dean Boyd, Dale E. Bauman, Deborah A. Ross, and Katherine D. Fagin, Dept. Animal Science, Cornell Univ., Ithaca, NY 14853 USA, and AMGen Co., Thousand Oaks, CA 91320 USA

Porcine somatotropin (pST) may repartition nutrients toward lean tissue growth by altering responsiveness of tissues to homeostatic signals. We examined this for insulin (I) using the euglycemic clamp. Eight crossbred barrows (65 kg) received daily injections (SQ) of pituitary pST (120 ug/kg bw) or excipient for 28 d. Pigs were cannulated (ear veins) for simultaneous infusion and sampling and fasted for 12 h (d 28) prior to the euglycemic clamp. After a priming pulse, I infusion rate was maintained at 14 ng/min/kg. Simultaneously, glucose (G) was infused at a rate to maintain basal concentrations of glucose, thereby providing a minimal estimate of G metabolized by tissues and a measure of I sensitivity. Treatment with pST resulted in an elevation ($P<.02$) of fasting basal whole blood G (76 vs 59 mg/dl) and plasma I (8.03 vs 2.80 ng/ml) and a dramatic reduction in the G infusion rate (3.16 vs 11.37 mg/min/kg, $P<.01$) required to maintain basal concentrations of G. Therefore, the G uptake by the tissues in response to elevated blood I was less in pST-treated pigs (28% of control). In pigs, pST both increases basal blood G and I levels, and decreases the I sensitivity of the tissues.

3

INTERRELATIONSHIPS BETWEEN PORCINE GROWTH HORMONE ADMINISTRATION (pGH) AND ENERGY INTAKE ON THE PERFORMANCE AND PROTEIN DEPOSITION OF GROWING PIGS.

Campbell, R.G.*, Steele, N.S., Caperna, T.J., McMurtry, J.P. and Mitchell, A.D., USDA, Agricultural Research Service, Beltsville, MD 20705 USA

Thirty-six barrrows were used to investigate the effects of pGH dose (USDA-pGH B1; 0 vs 100 $\mu g \cdot kg\ Bwt^{-1} \cdot d^{-1}$) and energy intake (EI; ad libitum, 5.9 and 4.8 Mcal $DE \cdot d^{-1}$) on the performance and protein deposition of pigs growing from 25 to 55 kg live weight. Although GH reduced ad libitum EI from 8.24 to 7.1 Mcal $DE \cdot d^{-1}$ ($P<0.01$) there were no significant interactions between the effects of pGH dose and EI on performance. Growth rate increased linearly with increase in EI and was improved by 25-28% by pGH ($P<0.01$). Feed:gain was reduced 20% by pGH ($P < 0.01$) but unaffected by EI. Body fat content at 55 Kg increased with increase in EI and was reduced by 30-34% by pGH ($P < 0.01$). Differences in growth performance and body composition between pigs administered 0.0 and 100 μg $pGH \cdot Kg\ Bwt^{-1} \cdot d^{-1}$ were associated with, and probably the direct result of, concomittant differences in protein deposition which was increased 50% by pGH. Results suggest GH is the major factor limiting rate of protein deposition during the pig's post-natal development. The action of pGH in stimulating protein deposition is independent of energy intake.

9

PATHOPHYSIOLOGY OF PLASMA GLUCAGON-LIKE IMMUNOREACTANT INCREASE IN COCCIDIA-INFECTED BROILER CHICKS

Allen, Patricia C., USDA-ARS, Animal Parasitology Institute, Beltsville, MD 20705 USA

Coccidial infections (Eimeria sp.) of the chick small intestine cause significant increases in plasma levels of glucagon-like immunoreactants (GLIs), of gut origin, from 3 through at least 8 days postinfection (PI). Decreased plasma lipids, altered plasma lipoprotein patterns and decreased growth also occur at this time. Fatty acid synthesis in the liver, growth rate and intestinal length are parameters which have been used to study possible pathophysiological actions of GLI. Fatty acid synthesis was inhibited by plasma from infected chicks, GLI-positive chromatographic fractions of extracts from infected intestines and by commercially available porcine GLI (P-GLI). Growth was numerically, but not significantly inhibited by multiple injections of P-GLI over a 4 day period. This treatment also significantly increased intestinal length, a phenomenon observed in coccidial infections. The effects on growth and intestinal lengths of extract fractions from infected intestines are currently being tested.

10

TESTOSTERONE 15α-HYDROXYLASE ($P\text{-}450_{15\alpha}$) EXPRESSION IN MOUSE LIVER AND KIDNEY IS REGULATED BY ANDROGENS.

E. James Squires* and M. Negishi
NIEHS/NIH, Research Triangle Park, NC 27709 USA

Testosterone 15α-hydroxylase ($P\text{-}450_{15\alpha}$) is a form of cytochrome P-450 purified from 129/J female mouse liver. $P\text{-}450_{15\alpha}$-dependent activity is expressed at more than 10 times higher levels in female liver than in male liver, while in the kidney the activity is 10 times higher in males compared to females. Following castration, the $P\text{-}450_{15\alpha}$ activity in the kidney decreased to about 50% while levels of $P\text{-}450_{15\alpha}$ in the liver rise to about 10 times that of controls. These differences are also found in levels of $P\text{-}450_{15\alpha}$ protein determined by Western blotting and P-450 mRNA determined from Northern blotting analysis. Administration of testosterone propionate to castrated males causes kidney levels of $P\text{-}450_{15\alpha}$ activity, protein and mRNA to rise and liver levels to fall to near normal values. Two cDNAs, designated Type I and Type II (corresponding to 15α-29 and 15α-15, JBC 260:15357-15361, 1985), were present in approximately equal amounts in a female liver cDNA library. They shared about 98% sequence homology but were distinguished from each other by several diagnostic restriction sites created by single base substitutions. In contrast to the liver, only Type II cDNA was present predominantly in female kidney and only Type I cDNA was predominant in male kidney. The effect of androgens on the expression of Type I and Type II genes in kidney and liver is under investigation.

11

GENE EXPRESSION OF ORITHINE DECARBOXYLASE IN DEVELOPING EMBRYOS OF MUSCA DOMESTICA

David Vaske*, Janet Strong+, Roger Leopold+, and Robert Sparks* Department of Biochemistry*, North Dakota State University and USDA Metabolism and Radiation Research Laboratory+, Fargo, ND

The rate limiting step in polyamine biosynthesis is the conversion of ornithine to putrescine by ornithine decarboxylase (ODC), a highly regulated enzyme with a rapid turnover rate. Further, cell proliferation and tissue growth have generally been found associated with increased intracellular polyamine concentration. The goal of our work is to understand the molecular control mechanisms involved in the regulation of ODC gene expression during embryonic development. We have determinined the specific activity of ODC and the quantity of ODC polyA-contining mRNA in developmentally synchronized embryos. Conversion of ^{14}C ornithine to $^{14}CO_2$ was used to assay ODC activitiy throughout development. We found that ODC activity was undetectable until 12 hrs into the developmental sequence. The acivity then sharply increased to a peak activity of 1 nmole CO_2 released/hr/mg protein at 13 hrs, the time of hatching. This increase in ODC activity corresponded to slightly decreased levels of polyamines in the developing embryos. Addition of 1 mM difluoromethyl ornithine, a potent inhibitor specific for ODC, to the reaction mixture completely eliminated the ODC activity. To monitor ODC mRNA synthesis during embryogenesis, riboprobes made from a mouse ODC cDNA clone were used in hybridization experiments on northern blots that contained polyA mRNA isolated from several developmental stages.

12

A NEW METHOD FOR ISOLATION OF BOVINE GROWTH HORMONE
Vitaly Spitzberg*, C. Mark Eppler#, and Barry Rosenblatt+. American Cyanamid Co.#, Princeton,NJ, 08540, Centocor Corp+., Glen Mills, PA, 19342, and Ithaca, NY* USA

The new method employs a selective extraction of growth hormone (bGH) from a 15,000 x g sub-cellular sediment of anterior pituitary gland with 130-150 mM NH_4HCO_3 (pH 7.2-7.4) at 26°C for 60 min., followed by ammonium sulfate fractionation and chromatography on DEAE- or CM-cellulose and Sephadex G-75. The method yields an exceptionally pure hormone in high yield; about 2 mg bGH/gm of pituitary gland. The isolated hormone can be crystallized by a batch method at low ionic strength and isoelectric pH. Sequencing showed the C-terminus to be intact, with 55% alanine and 45% phenylalanine at the N-terminus. The biological activity of this preparation was very similar to that of a well-characterized bGH standard in two assays, receptor binding and growth of hypox mice.

13

ELEVATED HEPATIC GROWTH HORMONE AND PROLACTIN RECEPTORS IN HUMAN GROWTH HORMONE TRANSGENIC MICE.
F.C. Leung*, Pacific Northwest Laboratory, Richland, WA, C.I. Rosenblum, and H. Chen, Merck & Co., N.J. Rahway, J.S. Yun and T. Wagner, Ohio University, Athens, OH.

The objective of this study is to examine the relationship of hepatic growth hormone (GH) and prolactin (PRL) in human growth hormone (hGH) transgenic mice. Recombinant DNA fragments containing the mouse metallothionein (mMT)-I promoter/regulator sequence (mMT) fused with the genomic coding sequence of the human GH DNA were microinjected into the male pronuclei of fertilized mouse eggs to generate MT-hGH transgenic mice. Stable incorporation and expression of the foreign fusion genes resulted in high GH mRNA, elevated circulating GH concentration, and increased growth rates. However, there was no apparent correlation between gene copies, circulating GH concentration and growth rate among the MT-hGH transgenic mice. We examined specific GH and PRL receptor bindings using ^{125}I-bovine GH and ^{125}I-ovine PRL as labeled ligands. All MT-hGH transgenic mice examined by radioreceptor assay had elevated hepatic GH and PRL receptor bindings. Specific hepatic GH and PRL receptor bindings were highly correlated with growth rate. These results demonstrate that hepatic GH and PRL receptors were significantly elevated in MT-hGH transgenic mice and suggested that elevated GH and PRL receptor binding may play an important role in the growth of these animals.

INSECTS 21 - 26

21
DEVELOPMENTAL CHANGES IN GALLERIA MELLONELLA STORAGE PROTEINS
Malone, Christine C.* & Donald L. Silhacek, USDA, ARS, Insect Attractants, Behavior, & Basic Biology Research Laboratory, Gainesville, FL 32604 USA

The wax moth, Galleria mellonella (L.), synthesizes an 82K (kilodalton) storage protein in the fat body, and releases it into the hemolymph during the larval stages. After this synthetic phase, it is resorbed by the fat body, concurrent with gut purge. The protein is then incorporated into protein granules in fat body cells. The occurrence of the 82K storage protein has been followed by quantitative immunoelectrophoresis, as well as electron microcopic immunocytochemistry. Ultrastructural stages in the fat body during the development from the last larval stadium were examined.

22
IN VITRO KINETICS OF ECDYSONE SECRETION BY GYPSY MOTH PROTHORACIC GLANDS IN RESPONSE TO TISSUE EXTRACTS AND PARTIALLY PURIFIED PROTHORACICOTROPIC HORMONE
*Thyagaraja, Belgaum S.†, Thomas J. Kelly, Edward P. Masler, Charles W. Woods, Robert A. Bell, *Richard B. Imberski, and Alexej B. Borkovec, *Dept. of Zoology, University of Maryland, College Park, MD 20742. †RSRS, Central Silk Board, C.R. Nagar, Karnataka, India. Insect Reproduction Laboratory, ARS, USDA, Beltsville, MD 20705 USA.

Ecdysteroid production by prothoracic glands of the gypsy moth, Lymantria dispar, was stimulated by exposure of the gland to neural tissue extract in vitro. A two-phase bioassay was developed for kinetic studies and isolation of the steroidotropic neurohormone, prothoracicotropic hormone (PTTH). Secretory kinetics were determined for stimulated and non-stimulated glands at each day of the last (5th) instar and secretory products were analyzed by RP-HPLC and ecdysteroid RIA. Ecdysone was the only ecdysteroid detected. Whole brain and retrocerebral complexes, extracted in Grace's medium or acidic methanol, demonstrated substantial in vitro PTTH activity. This activity was separated by size exclusion chromatography into two active molecular weight species. The activation kinetics of these two forms were compared and age-dependent PTTH activity was studied.

23
ISOLATION OF TWO NEUROPEPTIDES IN THE AKH/RPCH-FAMILY FROM DIPTERA.
Jaffe,* Howard, Raina, Ashok K., Hayes, Dora K., Lancaster, J. L., and Morgan, Neal O., USDA, ARS, Beltsville, MD 20705 and Dept. of Entomology, University of Arkansas, Fayetteville, AR 72701 USA

Two neuropeptides in the adipokinetic/red pigment concentrating hormone (AKH/RPCH)-family have been isolated and purified from the corpora cardiaca of Diptera in the Family Tabanidae. Both peptides were purified by sequential gradient elution or three reversed phase-high performance liquid chromatographic systems. Photodiode array UV spectroscopy indicated that both were tryptophan-containing peptides in the AKH/RPCH-family. Partial sequence information and preliminary data on the biological activity of these peptides will be presented.

24
ECDYSONE 3-EPIMERIZATION IN MANDUCA SEXTA
Weirich, G. F.*, M. J. Thompson and J. A. Svoboda, Insect and Nematode Hormone Laboratory, USDA-ARS, Bldg. 467, BARC-East, Beltsville, MD 20705 USA

The insect molting hormones ecdysone and 20-hydroxyecdysone are converted to the corresponding, hormonally inactive 3-epiecdysteroids by soluble enzymes of larval M. sexta midgut. This conversion requires oxygen and NADH or NADPH. If incubated under anaerobic conditions with a semipurified midgut enzyme preparation (Sephadex G-25-filtered high-speed supernate of midgut homogenates, G-25 sup) ecdysone is not metabolized. Incubations containing ecdysone or 20-hydroxyecdysone and G-25 sup, but no reduced cosubstrates, yield 3-dehydroecdysteroids instead of 3-epiecdysteroids. Incubations of G-25 sup with 3-dehydroecdysteroids and NADH or NADPH result in the formation of 3α- and 3β-hydroxysteroids in varying proportions depending on the cosubstrate added. With NADH the incubations yield mostly the hormonally inactive 3α-hydroxysteroid(s), with NADPH, mostly the hormonally active 3β-hydroxysteroid(s). Thus, the midgut of M. sexta contains at least three enzymes involved in the 3-epimerization of ecdysteroids: ecdysone oxidase, 3-oxoecdysteroid 3α-reductase(s) and 3-oxoecdysteroid 3β-reductase(s). This complicated enzyme system may provide multiple control points for the regulation of the molting hormone concentration.

25
BINDING OF SYNTHETIC AND NATURAL PEPTIDES TO COMPONENTS OF MEMBRANES FROM INSECTS TREATED WITH A CONTROLLED SUBSTANCE.
Hayes,* Dora K., Jaffe, Howard, Morgan, Neal O. and Redfern, Robert E., USDA, ARS, Beltsville, MD 20705 USA

Affinity chromatography of crude membrane preparations obtained from abdomens of the face fly, Musca autumnalis De Geer, on columns prepared using insulin, glucagon and a synthetic nonapeptide similar to peptides in the naturally occurring AKH/RPCH-family provided evidence for preferential binding of some proteins. Polyacrylamide gel electrophoresis and high performance size exclusion chromatography (HP-SEC) indicated that the molecular weight of the proteins bound was between 40 K and 105 K daltons. Differences were observed in electrophoretic properties of proteins isolated from membrane preparations obtained from flies fed a controlled substance in sugar water and similar proteins obtained from flies fed sugar water alone. Data suggest that feeding such materials may affect binding properties of membrane proteins.

26
THE INSECT GROWTH REGULATOR AZADIRACHTIN: EFFECTS ON NUTRITION AND DEVELOPMENT AND TIME COURSE OF EXCRETION IN HELIOTHIS VIRESCENS (FABR.) (TOBACCO BUDWORM)
Barnby, Mark A.* & James A. Klocke, NPI, University of Utah Research Park, 417 Wakara Way, Salt Lake City, UT 84108

The plant chemical azadirachtin (AZA) was administered to fifth instar Heliothis virescens. In artificial diet, AZA at 0.03125 ppm reduced consumption and weight gained by larvae. At 0.25 and 0.5 ppm, the efficiencies of conversion of digested and ingested food, respectively, were reduced, whereas digestibility increased at 0.25 ppm. AZA injected orally at 0.25 and 0.5 ug delayed molting to the pupal stage and produced defective pupae. Higher doses (1, 5, and 10 ug) completely inhibited pupation, and reduced whole-body titers of molting hormone, β-ecdysone. Lower amounts of AZA (0.3 ug) inhibited pupation when injected directly into the hemocoel. Tritiated dihydroAZA injected orally was excreted more slowly than when injected directly into the hemocoel. Otherwise, patterns of labelled-body tissues between larvae injected orally and into the hemocoel were similar. Our results suggest that AZA affects H. virescens similar to other species tested.

31
IDENTIFICATION OF A CALMODULIN-STIMULATED, (Ca^{2+}+Mg^{2+})-ATPase IN A PLASMA MEMBRANE FRACTION FROM MAIZE LEAVES

Curtis Robinson*and Thomas J. Buckhout
Plant Photobiology Laboratory, Agricultural Research Service, USDA, Beltsville, Maryland 20705

An investigation of ATPases, which may be involved in regulation of Ca^{2+} transport across the plasma membrane (PM), was conducted. PMs were isolated from 14-day-old maize leaves (Zea mays L.) by two-phase partitioning. The PM fraction was ca. 9-fold enriched in the specific activity for PM marker enzymes and showed minimal contamination from mitochondria, chloroplasts and endomembranes. Ca^{2+}/calmodulin stimulated the basal Mg^{2+}-ATPase level by 10-20%. The stimulation was dependent on Mg^{2+} and Ca^{2+} and maximal at 20 uM total Ca^{2+} (ca. 3 uM free Ca^{2+}). The stimulation was specific for Ca^{2+} with an activity series of Ca^{2+}>Sr^{2+}>Mn^{2+}>> Ba^{2+}>>Co^{2+}>Cu^{2+}. Stimulation was largely independent of the Ca^{2+} salt used. The calmodulin stimulation was saturated at 1 uM calmodulin. The calmodulin antagonist W7 inhibited the stimulation by 50% ca. 75 uM, while the less active analogue, W5, showed no significant inhibition at concentrations greater than 150 uM. Characteristics of the calmodulin stimulation would suggest a potential involvement of this ATPase in Ca^{2+} transport across the PM.

32
MAIZE ROOT PLASMA MEMBRANE ELECTRON TRANSPORT

Luster, D. G.*and T. J. Buckhout, Plant Photobiology Laboratory, USDA-Agricultural Research Service, Beltsville, MD 20705

Plasma membrane (PM) electron transport has been implicated in regulation of plant growth via redox-linked proton efflux into the apoplastic space. We are examining electron transport activities present in highly purified PM preparations from dark-grown maize roots. The plasma membrane fractions contain NAD(P)H:ferricyanide, NAD(P)H:duroquinone, and NAD(P)H:(semi) dehydroascorbate activities. Measurements of reductase activities utilizing right-side out plasma membrane vesicles revealed potential differences in orientation of the respective reductases within the plane of the membrane. NADH:ferricyanide has been purified 500-fold by solubilization with Triton-X-100 and affinity chromatography on Cibacron-blue F3GA agarose. The purified reductase preparation contained a prominent 42 Kd polypeptide and several minor bands when evaluated by SDS-PAGE. Further experiments to examine the orientation of redox components in the PM are in progress.

33
ORIGINS OF ABSCISIC ACID IN MAIZE EMBRYOS

J. D. Smith, Texas A & M University, Department of Soil and Crop Sciences, College Station, TX

Origins of Abscisic Acid in Maize embryos have been confounded by the fact that alternative biosynthetic pathways have been suggested and the possibility that ABA recovered from the embryo may have been synthesized in the maternal plant. Genetic and chemical inhibitors of carotenoid synthesis were used with both field grown and in vitro cultured kernels to elucidate this problem. Data are presented which indicate that ABA is synthesized via the carotenoid pathway, that ABA is synthesized in the maternal plant, cob tissue and the kernel itself, and that all three sources contribute to the total ABA in maize embryos.

34
COMPARISON OF THE ENDOGENOUS GIBBERELLINS IN THE SHOOTS AND ROOTS OF VERNALIZED AND NON-VERNALIZED CHINESE SPRING WHEAT SEEDLINGS

Jiann-Tsyh Lin* & Allan E. Stafford, WRRC, ARS, USDA, 800 Buchanan Street, Albany, CA. 94710

Endogenous gibberellins (GAs) in Chinese Spring wheat seedlings were isolated by high-performance liquid chromatography (HPLC) and identified by combined capillary gas chromatography-selected ion monitoring (GC-SIM). Gibberellins A_1, A_3, A_{19}, A_{20}, A_{44}, and A_{53} were identified in the shoots, A_{19} and A_{20} in the roots. The identification of these 13-hydroxylated GAs demonstrates the presence of the early-13-hydroxylation pathway in wheat seedlings. Based on peak area of total ion response of 5 characteristic ions by GC-SIM, the relative level of GAs in the shoots is GA_{44} > GA_{19} > GA_1 = GA_3 > GA_{20} for the non-vernalized wheat seedlings, and GA_{44} > GA_{19} > GA_{53} = GA_3 > GA_1 = GA_{20} for the vernalized wheat seedlings. The C_{20} GAs, GA_{53}, GA_{44} and GA_{19}, are present at higher levels in shoots of the vernalized (flowering) wheat seedlings than in the non-vernalized (rapidly growing) wheat seedlings. In contrast, levels of the C_{19} GAs, GA_{20}, GA_1 and GA_3 were lower in the shoots of the vernalized wheat seedlings than in the non-vernalized wheat seedlings. The conversion of GA_{19} to GA_{20} (C_{20} to C_{19} GAs) may thus be a regulatory step controlling the concentrations of the physiologically active GA_1 and GA_3 for stem elongation.

35
RESPONSE OF MORNINGGLORY CALLUS TO GLYPHOSATE: EFFECT OF CALLUS AGE AND FREQUENCY OF HERBICIDE EXPOSURE

Simpson, Sandra F.*& Susan N. Gilbey, FMC Corporation, Agricultural Chemicals Group, P.O. Box 8, Princeton, NJ 08543 USA

Morningglory (MoG) callus was initiated from seedling hypocotyl tissue on B5 (5 uM 2,4-D) in 1982. The initial callus was excised and has been maintained on B5 (0.5 uM 2,4-D) in the dark, 25 C, 3-wk subcultures. Two glyphosate-treated lines were derived from MoG. Line MoG-G1 was exposed to glyphosate once ca. 5 mo after callus initiation, while line MoG-G2 was exposed twice, first ca. 5 mo and then ca. 3 yr after callus initiation. The growth response to glyphosate exposure, calculated as I_{50} values, showed that all MoG lines became less sensitive to the herbicide as the callus cultures aged.

36
INDOLE-3-ACETIC ACID AND GIBBERELLIN CONTENTS OF HIGH AND LOW "VIGOR" APPLE BUDS AND LEAVES DURING ONE SEASON

Buta, J.G.*, Reed, A.N., Murti, G.S.R., and Francis, B.A. USDA/ARS, Plant Hormone Laboratory, Beltsville, MD.

Buds and leaves from shoots of the current year of high and low "vigor" or growth-rate apple trees were collected from April through July for hormone analyses. Indole-3-acetic acid (IAA) was analyzed by GC-MS using ^{13}C IAA as the internal standard. The IAA content of 52 ng/g FW in high vigor leaves in early May decreased to 9 ng/g FW in mid-June while 12 ng/g FW of IAA in low vigor leaves in early May was comparable to 8 ng/g FW in mid-June. The mid-April IAA content was 70 ng/g FW in high vigor buds and 60 ng/g FW in low vigor buds. The levels of hormone in bud tissue diminished with fluctuations through the time of the study. Some data on gibberellin (GA) contents were obtained by the dwarf rice bioassay. GA levels of ca 5 ng/g FW in both high and low vigor leaves were highest in early May when the high vigor buds contained 15 ng/g FW GA and the low vigor buds <1 ng/g FW. A correlation between vigor of growth and levels of IAA and possibly gibberellin appears to be supported by these preliminary findings.

37
LIGHT-STIMULATED GIBBERELLIN BIOSYNTHESIS IN *GIBBERELLA FUJIKUROI*

Stephen W. Johnson and Ronald C. Coolbaugh*
Department of Botany, Iowa State University, Ames, Iowa 50011

Gibberellic acid was originally isolated from the fungus *G. fujikuroi*. It was subsequently reported that light stimulates GA production in this fungus (Mertz and Henson, 1967, Physiol. Plantarum, 20: 178-199). We have recently confirmed and extended these results.

Liquid cultures of *G. fujikuroi* strain Gf-1A were grown on an orbital shaker at 250 rpm in the light or dark for six days. The filtrates from these cultures were analyzed for GAs while the mycelia were harvested for enzyme preparation. GAs were extracted from the filtrates with acidic ethyl acetate and partially purified using C_{18} reversed phase HPLC. The identity and quantity of GA_3 was confirmed by GC-MS after formation of the methyl ester trimethylsilyl ether. Soluble enzyme extracts were prepared by centrifuging the homogenized mycelia. The S_{150} enzyme preparations were incubated with ^{14}C-mevalonic acid, ATP, Mg^{++}, Mn^{++}, and the products were separated by TLC and quantified by liquid scintillation counting.

Our results indicate a 36-74% increase in GA_3 accumulation in the light. Furthermore, preliminary data from radiotracer studies indicate that the light-stimulated step(s) is in the early part of the pathway. Incorporation of ^{14}C-mevalonic acid into *ent*-kaurene is nearly 60% greater in preparations from light grown cultures than from those grown in the dark.

38
OVEREXPRESSION OF THE GENE INVOLVED IN CYTOKININ BIOSYNTHESIS WITH THE CaMV 35S PROMOTER ENHANCES SHOOT PRODUCTION ON TRANSFORMED TOBACCO AND CUCUMBER GALLS

Ann C. Smigocki* & Lowell D. Owens, USDA-ARS, Beltsville, MD 20705 USA

In order to study the effects of the ipt gene expression on plant development, we have made constructs of the ipt gene in which the gene is under the control of different promoters. These gene contructs were cloned into a binary plasmid vector which also carries the gene for kanamycin resistance. Wounded stems or hypocotyls were infected with A. tumefaciens carrying the binary plasmid vector and an avirulent Ti plasmid. In cucumber, larger galls were produced when the ipt gene was under the control of a strong, constitutive promoter, the cauliflower mosaic virus 35S (CaMV 35S) promoter, and only on these galls shoots were observed. In tobacco, the time required for production of shoots on wounds decreased as the strength of the promoter on the ipt gene increased. The sizes and numbers of shoots on tumors were also indicative of the infecting strain. These experiments demonstrate that the morphogenic potential of a transformed plant cell can be enhanced by increasing the promoter strength of a gene directing the synthesis of cytokinin.

39
APPEARANCE OF 24,000 MW PROTEIN DURING TOMATO FRUIT RIPENING

Peiser* Galen, Gretchen King, Charles Hussey & Victoria Turner
NPI, 417 Wakara Way, Salt Lake City, UT 84108

A protein of 24,226 MW (NP24) previously found to be induced in NaCl stressed tomato tissue cultured cells and whole plants (Plant Molec. Biol. 7:441-449, 1986), also accumulates during ripening of 'Sierra Sweet' tomato fruit. As evaluated by immunoblot analysis, NP24 begins to increase in fruit at the mature green stages 2 and 3, and continues to increase as ripening progresses. The greatest amount of NP24 is detected in red fruit. Though NP24 is not detectable in immature green fruit, it can be induced in these fruit upon exposure to ethylene and this induction by ethylene is suppressed by norbornadiene.

NP24 accumulates during the normal growth of tissue cultured tomato cells; however, ethylene does not appear to control this accumulation. Elevated ethylene levels generated from 1-aminocyclopropane-1-carboxylic acid did not cause an increase in NP24 and silver thiosulfate, which blocks ethylene action, had little or no inhibitory effect upon its accumulation.

40
IMMUNOLOGICAL DETECTION OF ISOZYMES OF ALCOHOL DEHYDROGENASE FROM ARMILLARIA MELLEA FOR STUDIES OF THEIR RELATIONSHIP TO RHIZOMORPH DEVELOPMENT

Cohen, Susan D.* and Jerome J. Motta, Department of Botany, University of Maryland, College Park, MD 20742 USA

Armillaria mellea, a basidiomycete fungus, exhibits an unusual mycelial differentiation switch from individual mycelial filaments to an organized pseudoparenchymatous mycelial structure known as a rhizomorph. This rhizomorph functions as an infection structure during the life cycle of this fungal pathogen. A synchronized growth scheme was devised to observe rhizomorph differentiation. This morphological change is triggered by a minute amount of ethanol. The appearance of the enzyme, alcohol dehydrogenase (ADH) is correlated with the initiation of the events leading to rhizomorph formation. Multiple isozymes of ADH were detected by histochemical staining on native polyacrylamide gels. The presence of ADH in Armillaria mellea was further confirmed by immuno-dot blots with a polyclonal antisera produced to yeast ADH. The polyclonal antisera recognized 50 ng of yeast ADH at 1/100,000 dilution. ADH was also detected in SDS extracts of undifferentiated A. mellea at 1/50 dilution and the differentiated rhizomorph stage of A. mellea at 1/100 dilution. This antisera has also proven useful for Western blotting and is being used to further characterize the role of ADH isozymes during rhizomorph differentiation.

41
EMBRYONIC ABA LEVELS AND SENSITIVITY IN MATURING WHEAT GRAIN UTILIZING A MONOCLONAL ANTIBODY IMMUNOASSAY FOR ABA.

Mary Walker-Simmons*, USDA-ARS, Washington State University, Pullman, WA 99164-6420

An indirect enzyme-linked immunosorbent assay (ELISA) for quantitative analysis of abscisic acid (ABA) in wheat grain has been developed. With this assay +ABA amounts as low as 5 pg can be detected in crude wheat extracts. Utilizing the assay ABA levels in the embryo and seed remnant have been measured throughout wheat grain maturation. Additionally the sensitivity of maturing wheat embryos to ABA, as measured by the capability of ABA to block embryonic germination, has been determined. A comparison of dormant and non-dormant wheat grain shows little differences in endogenous ABA levels, but large differences in embryonic sensitivity to ABA.

42
BIOLOGICAL AND CHEMICAL EVALUATION OF NATIVE MISSISSIPPI PLANTS.

James D. McChesney* and Hiranthi Jayasuriya, Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677.

Biological evaluation of common Mississippi plants for potentially useful activity led to the discovery of Hypericum Drummondii, a common weedy plant, as having significant antimicrobial activity. A discussion of the screening procedures and further chemical investigations of active plants as exemplified by H. Drummondii will be presented.

43
STUDIES OF CA-CALMODULIN AS A SECOND MESSENGER FOR CYTOKININS IN GROWTH AND CHLOROPHYLL FORMATION

Zhifan Zhao* and Cleon W. Ross, Department of Botany, Colorado State University, Fort Collins, CO 80523

Cytokinins have been shown to act via Ca-calmodulin, a second messenger in plants and animals. While studying mechanisms of cytokinin promotion of growth and chlorophyll formation in excised cucumber (Cucumis sativus L.) cotyledons, a hypothesis that the the cytokinin-induced responses are mediated via Ca-calmodulin was tested in three ways: (1) Exogenous $CaCl_2$ and compounds that influence the cytosol free Ca^{2+} concentration were added to growth media to learn if the two processes are affected. (2) Several Ca-calmodulin inhinbitors were added to growth media to learn their effects. (3) Studies were made to determine if zeatin activates NAD kinase, an enzyme that converts NAD^+ to $NADP^+$ and is regulated by Ca-calmodulin; this was done by measuring the levels and ratios of nicotinamide coenzyme activities in crude extracts form zeatin-treated cotyledons. Major findings were: (1) $CaCl_2$ substantially decreased the zeatin effects at high concentrations. (2) Zeatin and light caused substantial increases in NAD^+ levels; and (3) Extraction of nicotinamide coenzymes from fresh cotyledons is simpler and yet more accurate that from cotyledons frozen in liquid N_2 or frozen then freeze-dried. Collectively, these results did not meet the criteria of Ca-calmodulin as a second messenger for cytokinins and the hypothesis proposed earlier is tentatively rejected.

1

GENETIC ANALYSIS OF CELLULAR DIFFERENTIATION IN YEAST: CELL CYCLE AND MEIOSIS

Simchen, G., Department of Genetics, The Hebrew University, Jerusalem 91904, ISRAEL

Saccharomyces cerevisiae, a unicellular organism, has two cellular differentiation processes which are similar to those found in almost all other eukaryotes, namely the cell cycle and meiosis. The cell cycle serves to propagate cells asexually, whereas meiosis generates the gametes and serves as the major means of eukaryotic sexuality.

Mutations affecting various aspects of the cell cycle and of meiosis have been obtained, affecting either aspects which are unique to one process, or common functions, or the choice between the two. Many of the corresponding genes have been cloned.

Differentiation of vegetatively growing cells into meiosis depends on the cells' diploidy and on starvation. Diploidy regulates meiosis via the mating-type gene system: MATa1 and MATα2 together repress RME1, which represses IME1, which is an inducer of meiosis. The latter gene needs also to be induced by starvation, via the adenylate cyclase system and the cAMP-dependent protein kinases; hence it may have a role in joining the two regulatory pathways which lead to meiotic differentiation.

2

REGULATION OF GENE EXPRESSION BY INDOLEACETIC ACID IN PEA EPICOTYL TISSUE.

Athanasios Theologis, Plant Gene Expression Center, USDA-ARS, Albany, CA 94710 and Department of Molecular Plant Biology, U.C. Berkeley, Berkeley, CA 94720 USA

The mechanism of induction of gene expression by auxin is poorly understood. Expression of the IAA4/5 gene in pea epicotyl tissue is detected 5 min after addition of IAA. This lag period is unaffected by protein synthesis inhibitors and is extended by 1) low IAA concentration, 2) the presence of cuticle, and 3) less active IAA analogs.

Expression of this gene is also induced by protein synthesis inhibitors. This induction has a lag period of 20 min.; protein synthesis inhibition is completed in 5 minutes. Gene expression begins to be induced when protein synthesisis is inhibited 70%; half maximal induction occurs at 85% inhibition. The mRNA is labile, with a $t_{1/2}$ of 60 min. that is not affected by the presence of IAA. Protein synthesis inhibitors, on the other hand, stabilize the mRNA, resulting in an enhanced rate of mRNA accumulation induced by auxin. These results suggest that IAA regulates gene expression at the level of transcription initiation. Protein synthesis inhibitors have a dual effect: they activate transcription by preventing the synthesis of a labile negative regulatory protein and they stabilize the mRNA.

3

VARIANT FORMS OF GROWTH HORMONE

U.J. Lewis*, The Whittier Institute for Diabetes and Endocrinology, 9894 Genesee Avenue, La Jolla, CA 92037.

Although it is now well established that there is not just one form but multiple forms of growth hormone, physiologic need for this array of substances is completely unknown. Besides the normal gene product, there are forms resulting from alternative mRNA splicing, and co- or posttranslational alteration. There is a second gene for hGH but its expression product has not yet been found in the pituitary gland. Alternative mRNA splicing and posttranslational modification of this second gene product may also exist. For all forms, posttranslational alterations include disulfide dimer formation, acylation of the amino terminus, deamidation, proteolysis, and very likely glycosylation. Artifactual alterations can arise during isolation of the various forms, such as oxidation of methionine, and structural analysis can identify these.

The physiologic need for the multiple forms can only be speculated upon. GH exerts many biologic effects, some of which are counter-regulatory, such as insulin-like and anti-insulin actions. This multiplicity of actions may require alterations of molecular structure to direct specific cellular reactions.

4

GENETIC COMPLEXITY OF OOGENESIS

Mahowald, Anthony P., & Brian Oliver, Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, OH 44106 USA

A complex array of genetic functions are necessary for production of properly functioning eggs. Utilizing genetic approaches, we have identified the major classes of developmental functions occurring during oogenesis and we have studied the interaction of this maternal information with zygotic genetic function. Three major groups of genetic loci will be discussed: maternal effect genes whose only activity is during oogenesis; maternal effect genes whose function can be supplied also during embryonic development; essential genes which are also required during oogenesis and/or embryogenesis. Both the overall properties and specific examples of genes belonging to each class will be presented.

5

HOMEO BOX-ROLE IN EMBRYONIC DEVELOPMENT

McGinnis, William J., Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, CT 06511, USA

Homeotic and segmentation genes of Drosophila effect crucial patterning decisions in the morphogenesis of the fruit fly body plan. Many of these genes are members of a highly diverged multi-gene family. The signal homology for this family is the homeo box, a protein coding sequence of approximately 180 base pairs. The mouse and human genomes also contain multi-gene families with homeo boxes very similar to those found in Drosophila A tenable hypothesis is that the various members of this gene family perform similar morphogenetic programming functions in both fly and mouse development.

Thus far, there are two lines of experimental evidence that support this hypothesis. The first is that comparison of specific Drosophila and mouse gene homeo box gene sequences shows that some of the individual genes in the two species are true homologues in terms of structure, i.e., individual homeo box genes had evolved conserved and separate functions before the evolutionary divergence that eventually gave rise to arthropods and mammals. The second line of evidence is that the patterns of expression of mouse homeo box genes during embryonic development show remarkable similarities to the patterns of expression exhibited by their Drosophila counterparts.

6

GENE TRANSFER FOR INCREASED ANIMAL GROWTH

R.E. Hammer[1], V.G. Pursel[2], C.E. Rexroad Jr.[2], C.A. Pinkert[1], D.J. Bolt[2], R.J. Wall[2], R.D. Palmiter[3] and R.L. Brinster[1] [1]University of Pennsylvania, Philadelphia, PA 19104, [2]USDA, Agricultural Research Service, Beltsville, MD 20705 and [3]University of Washington, Seattle, WA 98195.

Transgenic mice which contain growth hormone (GH) or a human growth hormone-releasing factor (hGRF) fusion genes exhibit enhanced growth. Mice containing the metallothionein promoter/regulator (MT) fused to either the rat, human (h) or bovine (b) GH gene exhibit metal inducible levels of GH mRNA, and have substantial quantities of foreign GH. Mice which contain a GRF fusion gene exhibit enhanced growth due to stimulation of endogenous GH synthesis and release. Transfer of GH fusion genes has been extended to rabbits and GH and GRF fusion genes to pigs and sheep. Transgenic pigs and sheep expressing genes consisting of the MT promoter fused to either the hGH or bGH genes or the GRF gene have been produced. Expression of hGH or bGH genes in pigs has not improved growth performance. However, hGH and bGH exert varied biological effects. Founder transgenic pigs have transmitted the gene and one line has been bred to homozygosity with respect to the transgene. Transgenic sheep which express a bGH fusion gene have not exhibited enhanced growth. Transgenic pigs and sheep containing hGRF genes are being examined for the consequences of transgene expression.

7
GENE INSERTION: ROLE AND LIMITATIONS OF TECHNIQUE IN FARM ANIMALS AS A KEY TO GROWTH

Caird E. Rexroad, Jr.* and Vernon G. Pursel, USDA, Agricultural Research Service, Reproduction Laboratory, Beltsville, MD 20705 USA

Structural genes for bovine (bGH) and human (hGH) growth hormones and human growth hormone releasing hormone (hGHRH) were ligated to the promoter for mouse metallothionein I and microinjected into the nuclei of embryos resulting in "transgenic" pigs and sheep. Efficiency of microinjection was low (0.1 to 0.9% of injected embryos) resulting in transgenic young of which 60% actually produced the gene product. Concentration of bGH and hGH in plasma varied greatly among animals and was unrelated to number of copies of the gene. Growth rates were not enhanced in transgenic pigs with elevated growth hormone; however, subcutaneous fat content was dramatically reduced. Transgenic male pigs successfully transmitted the gene construct to their progeny; however, expressing transgenic females do not exhibit normal estrous cycles. Genes used in these experiments were not readily regulated by heavy metal supplementation. Lambs with hGHRH had a low rate of expression and did not exceed controls for gain or feed efficiency. A single expressing lamb was refractory to exogenous hGHRH challenge. Before the full potential of gene transfer can be realized for regulation of growth of farm animals, promoter/regulator sequences that will permit full control of gene expression must be found.

8
PEPTIDE PRODUCTION AND STIMULATION OF NEUROENDOCRINE (NE) CELLS AND TUMORS OF THE LUNG

Gazdar, A.F., Oie, H., Cuttitta F., Nakanishi, Y., Linnoila, R., and Mulshine, J. National Cancer Institute and Naval Hospital, Bethesda, MD 20814 USA

We have used lung cancer as a model to study peptide production and growth stimulation of lung cancer cells. We have developed serum free fully defined media for the establishment and maintainence of most forms of lung cancer, as well as some of their precursor cells. Certain tumors of the lung, especially small cell carcinoma (SCLC), express markers characteristic of NE cells including presence of dense core secretory granules and multiple peptide hormones. The peptide most frequently secreted is related to amphibian bombesin or its mammalian homologue gastrin releasing peptide (BN/GRP). BN/GRP acts as an autocrine growth factor for SCLC and inhibiting its action by a specific anti-peptide monoclonal antibody decreases SCLC growth. Recently we have adapted many human tumor lines to replicate continuously in RPMI-1640 medium without any additives. This forces the tumor cells to secrete all of the factors essential for growth. Identification of autocrine growth factors offers several therapeutic strategies to prevent tumor replication.

9
GENES SPECIFYING CYTOKININ BIOSYNTHESIS IN *AGROBACTERIUM TUMEFACIENS*: REGULATION OF EXPRESSION BY PLANT PHENOLICS

Morris, Roy O., Gary K. Powell, Linda A. Castle & Norman G. Hommes. Dept. of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331 USA.

Agrobacterium tumefaciens contains two genes which code for enzymes capable of catalyzing cytokinin biosynthesis. The first *(tmr)* is located within the T-region and encodes a prenyl transferase (dimethylallylpyrophosphate:5'-AMP transferase) capable of synthesizing iPA 5'-phosphate. A similar gene *(tzs)* is present only on nopaline plasmids and is located close to the vir region. Extensive homology exists between these genes and a cognate *(ptz)* present in Pseudomonas savastanoi. Expression of *tzs* (but not *tmr* or *ptz*) is enhanced by certain plant-derived phenolics. Secretion of zeatin by the bacteria was induced >100-fold by acetosyringone and other phenolic compounds secreted by plants in response to wounding. Because stimulation of zeatin secretion is observed in *A. tumefaciens* strains bearing tzs but not in *E. coli*, other factors encoded by the Ti plasmid appear to be involved in control of expression. A *tzs*-containing plasmid was introduced into several *Agrobacterium* strains and appeared to alter tumorigenicity of some strains on some hosts. (Supported by Grants PCM 83-03371 from NSF, and 83-CRCR-1-1249 & 83-CRCR-1-1645 from USDA).

10
NEUROENDOCRINE REGULATION OF INSECT DEVELOPMENT,

W.E. Bollenbacher & T.R. Flanagan, Biology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514 USA

Insect development is characterized by a series of post-embryonic growth cycles, each culminating with a cuticular molt. Towards the end of each cycle, tissues respond to a complex hormonal millieu and become committed to express the cellular products of their future developmental stage. This hormonal millieu is largely specified by hormones released from two types of endocrine glands; the ecdysone producing prothoracic glands, and juvenile hormone producing corpora allata. Quantitative and qualitative features of the hormonal environment defined by the activities of these glands are largely under neuroendocrine and interendocrine control. As expected, a complex circuit of feedback loops integrates these systems. Specific examples of these developmental endocrine circuits are illustrated with our work on the tobacco hornworm, Manduca sexta.

11
EFFECT OF INHIBITORS OF JUVENILE HORMONE METABOLISM IN INSECT DEVELOPMENT

Hammock, Bruce D., Departments of Entomology and Environmental Toxicology, University of California, Davis, CA 95616 USA

The development of environmentally acceptable methods of insect control remains critical to agricultural productivity and profitability in developed and developing countries. In this laboratory we are attempting to exploit knowledge of endocrine regulation of larval-pupal transformation in the Lepidoptera to illustrate new paradigms for the development of insect control agents. One approach employed transition state theory to design powerful inhibitors of an esterase which specifically removes insect juvenile hormone and initiates pupation. Inhibition of this enzyme retards pupation. These inhibitors were used as affinity ligands to purify the enzyme. Injection of the enzyme disrupts normal insect development so methods to elicit precocious expression of the enzyme represents a second approach to developing control agents.

12
CHEMICAL AND GENETICAL MANIPULATION OF GIBBERELLIN CONCENTRATION IN PLANTS

Hedden, Peter & John R. Lenton, University of Bristol, Department of Agricultural Sciences, Long Ashton Research Station, Long Ashton, Bristol, BS18 9AF, UK

The gibberellins (GAs) constitute a very large group of tetracyclic diterpenoid carboxylic acids which are present in plants and some fungi. Work with GA-deficient dwarf mutants of several species has indicated that one, or a small number, of the GAs act as internal regulators of plant growth and development. Despite the current contoversy over the relevance to plant development of changes in the concentration of GAs and the other phytohormones, there is considerable interest in mechanisms whereby plants regulate GA concentration. At the same time an understanding of the mode of action of GA growth regulators at the tissue, cellular and molecular levels is being actively pursued. In this paper we will describe current work with synthetic growth retardants which depress endogenous GA levels and mutants with altered GA status, and discuss possible uses of these systems for examining GA action, their metabolic regulation and their role in the control of plant growth and development.

13
BIOSYNTHESIS OF ETHYLENE IN PLANTS AND ITS REGULATORY MECHANISM
Hidemasa Imaseki, Fac. Agricul., Nagoya Univ., Chikusa, Nagoya 464, JAPAN

Ethylene production rate in higher plants quickly changes during the life cycle of plants. This quick change results from rapid induction of the rate limiting enzyme of the biosynthetic pathway and rapid inactivation of the enzyme. ACC synthase has been shown to be the rate limiting enzyme, and auxins and tissue wounding, which stimulate ethylene production, specifically induce formation of ACC synthase which is quickly inactivated within cells with an apparent half life of 25 min. Thus, when synthesis of ACC synthase is slowed or stopped, the ethylene production rate quickly slows or stops., Synthesis of the enzyme is also controlled by other plant hormones such as cytokinin, abscisic acid and ethylene. In order to understand the regulatory mechanism of synthesis of the enzyme at the molecular level, we have tried to purify the enzyme from auxin-treated mung bean stems, but this has been unsuccessful because of unusual instability of the enzyme. Instead, we were able to purify wound-induced enzyme from winter squash mesocarp and to raise specific antibody. Immunochemical analysis of western blots of crude proteins and fluorography of n vitro translation products of poly(A)RNA indicated that the enzyme protein and mRNA are accumulated by the wound stimulus. The regulatory mechanism of ACC synthase induction will be discussed.

14
MASS SPECTROMETRY, GENETICS AND BIOCHEMISTRY: UNDERSTANDING THE METABOLISM OF INDOLE-3-ACETIC ACID.
Cohen*, Jerry D., Janet P. Slovin, Krystyna Bialek, Kai Hsien Chen, and Myra K. Derbyshire. USDA/ARS Plant Hormone Laboratory, Beltsville Agricultural Research Center-West, Beltsville, Maryland 20705 and Department of Botany, University of Maryland, College Park, Maryland 20742.

It is the goal of our laboratory to understand the processes that plants use to regulate the level of the naturally occuring auxin, indole-3-acetic acid (IAA). Three basic approaches have been taken that, when applied together, will yield information on the mechanisms and reactions involved in the regulation of the level of this phytohormone.

1. We have developed techniques for the analysis of IAA and related metabolites using GC-MS and stable isotopes for quantitation and in vivo analysis of metabolic reactions.

2. Using chromatographic and spectral analysis we have isolated and identified a variety of conjugates of IAA and have shown these to be important in the regulation of IAA levels. Most unique has been the isolation of a 3 kD peptide with IAA in amide linkage that is the major conjugate in extracts from bean seeds.

3. We have applied genetic techniques in order to extend and refine our metabolic studies. A mutant of Lemna that accumulates large amounts of IAA has been isolated and selections are being attempted to obtain mutants unable to hydrolyze IAA conjugates.

15
HORMONAL CONTROL OF GENE EXPRESSION DURING CEREAL EMBRYOGENESIS
Quatrano, Ralph S*., James C. Litts, John D. Williamson, Barbara Ballo, Greggory W. Colwell, Randy L. Chakerian & Roswitha Hopkins, Dept. Botany, Oregon St. Univ., Corvallis, OR 97331 USA

Our major goal is to understand the physiological and molecular controls operative in the expression of gene sets during cereal embryogenesis. The growth regulator abscisic acid (ABA) prevents cereal embryos from germinating but promotes normal embryo maturation when young embryos are cultured in vitro. The pattern of ABA accumulation in vivo and the ABA response in culture support its role as a natural regulator of development. Several of the proteins that appear to be regulated by ABA have been characterized and include the lectin wheat germ agglutinin and several storage proteins. The characterization and pattern of accumulation of these proteins and their mRNA's during grain development and in culture with ABA will be discussed. Special emphasis will be given to the mRNA's for the storage proteins and some characteristics of their gene sequences. Our data suggests that one of the ABA effects on embryogenesis in cereals is at the level of modulating mRNA levels, increasing those mRNA's essential for embryo maturation while decreasing those associated with germination. The effect is at both the transcriptional and post-transcriptional levels. These results with ABA on morphogenesis in developing cereal embryos will be discussed in view of the broader question of hormonal mechanisms in plants.
*Address: Du Pont Exp. Station, E402. Wilmington, DE 19898

16
MECHANISMS BY WHICH PORCINE SOMATOTROPIN ENHANCES PIG GROWTH PERFORMANCE.
Etherton, Terry D., Dept. of Dairy and Animal Science, The Pennsylvania State University, University Park, PA 16802.

Previous studies have domonstrated that porcine somatotropin (pST) markedly increases pig growth performance. This has created interest in developing a pST-based product and gaining a better understanding of the mechanisms by which pST increases growth rate, improves feed efficiency and alters carcass composition. We have found that pST decreases adipose tissue growth rate by suppressing lipogenic rate and blunting the insulin responsiveness of adipocytes. The decrease in lipogenesis is a chronic effect and is associated with a marked decrease in activity of several lipogenic enzymes. The pST-induced increase in muscle growth is associated with an increase in insulin-like growth factor I (IGF-I) concentration in plasma. We have purified a subunit of the carrier (binding) protein which transports IGF-I in pig plasma. This protein appears to play a role in transendothelial transport of IGF-I from plasma to the target tissue. We have also identified strategies which increase the effect of pST on growth performance. Collectively these results indicate that a better understanding of pST action will lead to ways to further enhance the effectiveness of pST in stimulating growth performance. The benefit of this to animal agriculture will be discussed.

17
HORMONAL AND NEURAL CONTROL OF FOOD INTAKE, SATIETY, EATING BEHAVIOR PATTERNS AND BODY WEIGHT
Sarah F. Leibowitz, The Rockefeller Univ., New York, N.Y. 10021

Animal studies have provided us with valuable information concerning potential endocrine and neurochemical substrates of normal and abnormal eating patterns. Through microinjection and lesion experiments, we have discovered that different brain areas respond in unique ways to different neurotransmitter substances. Very dramatic alterations in food intake, meal patterns, and appetite for specific foods can occur with neurochemical stimulation or lesions of particular brain sites, and evidence indicates that these effects reflect these sites' normal physiological function. These animal studies have permitted us to explore the nature of the interaction and the interconnections between multiple transmitter systems in specific brain areas and also between these brain systems and various endocrine/metabolic processes of the body. Specifically, we have established the existence of an α_2-noradrenergic receptor system of the medial hypothalamus, which stimulates meal size and eating rate, specifically for carbohydrate, while suppressing protein intake. This system, which is involved in energy homeostasis and is mimicked by pancreatic polypeptides, appears to be unique in its positive interaction with circulating corticosterone and glucose. A serotonergic receptor system of the medial hypothalamus directly antagonizes the effects of the α_2-noradrenergic system, while the opiate peptides stimulate appetite for fat and possibly protein, once again in association with adrenal hormones. These results have permitted us to formulate various animal models of eating disorders and of drug action.

18
PATTERNS OF PHYTOCHROME-INDUCED GENE EXPRESSION IN ETIOLATED PEA BUDS
Thompson, W. F.[1], L. S. Kaufman[2], A. Sagar[3], B. Horowitz[3] and W. R. Briggs[3], [1]Depts. of Botany and Genetics, North Carolina State University, Raleigh, NC 27695, [2]Dept. of Biology, University of Illinois, Chicago, IL 60680, and [3]Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA 94305 USA

Transfer of dark grown pea seedlings to white light initiates rapid growth of leaf primordia and development of the photosynthetic apparatus. This process is potentiated by previous brief illumination with low intensity red light acting through the phytochrome system. Stimulation of the phytochrome system increases the concentration of several different cytoplasmic mRNAs. Although these changes occur in response to the same light stimulus acting through the same photoreceptor, certain transcripts have much lower red light fluence requirements for induction than other transcripts. Different transcripts also respond differently in experiments which measure the length of time required to complete the stimulus transduction process, and may also exhibit different time courses of accumulation. In addition, some responses involve increases in both nuclear and cytoplasmic transcripts while in other cases only cytoplasmic mRNA levels change significantly. The response diversity we observe is evidence that several different signal transduction pathways are operative in regulating the levels of different mRNAs and suggests that the molecular mechanisms by which light affects gene expression differ for different genes.

19
OLIGOSACCHARIDE SIGNALING IN PLANTS
Clarence A. Ryan. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164.

Fragments of fungal and plant cell walls including β-glucans, chitin and chitosan fragments and oligogalacturonides have been shown by a number of researchers to act as signals to activate plant defensive responses. Oligogalacturonide fragments can be generated from plant cell walls by the action of PGases and PG-lyases secreted by fungi and bacteria, and by PGases already present in plants. Our research on the role of oligouronide fragments in the wound induction of proteinase inhibitor genes in leaves of tomato plants has shown that α-1,4-oligouronides as small as the dimer can activate these genes. The unsaturated dimer, a product of PG-lyase, is also a potent inducer of proteinase inhibitor genes. The role of oligosaccharides as possible systemic signals in activating transcription of proteinase inhibitor genes from defense against insects will be discussed. (Supported in part by grants from the USDA and NSF.)

20
FEEDSIDEWARD MECHANISMS COORDINATING HORMONE CONCENTRATION: DELTA (δ) RHYTHMS. Halberg, F., Wu, Y., Cornelissen, G.S., Sanchez de la Pena, S. and Hayes, D.K.,* Lab. Medicine Dept., Univ. of Minn., Minneapolis, MN 55455 and *USDA, ARS, Beltsville, MD 20705 USA

Environmental schedule shifts can be beneficial, neutral or harmful. Outcomes depend upon the network of the body's intermodulating ∿ 24-hour (circadian), ∿7-day (circaseptan) and ∿ half-weekly (circasemiseptan) rhythms, including spontaneous (α), reactive (β), modulating (γ) and frequency dividing (δ) rhythms, modeled ex vivo by pineal, pituitary and adrenal interactions. With gland harvest from rodents kept on staggered lighting regimens, isophasic and heterophasic gland preincubations followed by incubations reveal predictably rhythmic interactions consisting of inhibition, no effect and stimulation at the level of hormone (corticosterone) production, underlying similar predictable, directionally different, effects at the cellular level, gauged by DNA synthesis. Rhythm scrambling by environmental schedule shifts can be thus exploited to manipulate and optimize, with survival, growth in health, e.g., in the giant green alga, Acetabularia mediterranea, and to prevent malignancy, e.g., in CD2F1 mice developing a spontaneous breast cancer. δ rhythm patterns are of interest for the optimization of the "last cell kill" in cancer immuno- or chemotherapy or of the "last insect kill" by pesticides in agriculture.

21
REGULATION OF FETAL GROWTH: IMPORTANCE TO GROWTH AND TISSUE DEPOSITION IN ANIMALS. Dennis R. Campion* and Gary J. Hausman. USDA-ARS, Russell Research Center, Athens, GA. 30613.

We have highlighted the two models (fetal hypophysectomy and obesity) to illustrate that: 1) the histochemical and biochemical differences associated with hypophysectomy do not express themselves until 72-74 days of gestation and that myonuclear proliferation (DNA content and satellite cell content) are not influenced. Thus, muscle DNA and fiber number are not under neural or hormonal (endocrine) regulation after 45 days of gestation; secondly, 2) in the obese models, the genetic difference from lean controls in fiber number must be regulated by events occurring before 70-90 days of gestation; 3) paracrine or autocrine regulation of fetal muscle growth is indicated by the similarity in serum proliferative activity across ages and fetal models; 4) since preadipocytes respond differently to sera from these fetal models in terms of proliferative activity, these cells are under different developmental controls. Thus, adipose tissue is relatively more responsive to extra-tissue stimuli while muscle is relatively more resistant to such stimuli. To extrapolate further, these data imply one could influence fat cell number independent of an effect on myogenesis.

22
RUMINANT SPLANCHNIC TISSUES - ENERGY COSTS OF ABSORPTION AND METABOLISM.
Huntington, G.* & McBride, B., USDA, Agricultural Research Service, Beltsville, MD 20705 USA & Univ. of Guelph, Ontario, Canada.

Ruminant splanchnic tissues (portal-drained viscera [PDV] and liver [L]) are 6-13% of body tissue (BT) mass yet account for 40-46% of BT energy lost as heat (HE). We will describe processes involved in this disproportionately high HE from PDV and L. HE is calculated from oxygen uptake and metabolite flux in vivo and vitro. HE from PDV is 17-25% of BT HE. Major sources of HE from gut mucosa are: Na,K-ATPase activity; protein synthesis; and protein degradation (28-61, 20, and 14%, respectively, of mucosal HE). Other mucosal sources of HE (maximally 38% of HE) include DNA/RNA flux, Ca transport, membrane phospholipid flux and H-ATPase activity. L (3% of BT) accounts for 22-25% of BT HE. Major sources of HE from L are: Na,K-ATPase activity; protein synthesis; substrate cycling; and urea synthesis (22-55, 16-24, 18-24, and 25%, respectively, of L HE). Other L sources of HE (maximally 29% of L HE) include gluconeogenesis, DNA/RNA flux, protein degradation and membrane phospholipid flux. Assuming on average PDV and L each account for 20% of BT HE, major metabolic costs of PDV and L (% BT HE) include Na,K-ATPase activity (10%), protein synthesis (7%), protein degradation (2%), substrate cycling (4%) and urea synthesis (5%).

23
INFLUENCE OF EARLY PLANE OF NUTRITION ON ENZYME SYSTEMS AND SUBSEQUENT TISSUE DEPOSITION.
McMurtry, J.P., Rosebrough, R.W., Plavnik, I., and Cartwright, A.L. USDA, Agricultural Research Service, Beltsville, MD 20705 USA

The occurrence of compensatory growth is of great significance because of its potential effects on body composition (muscle versus adipose development) at maturity. Studies have been conducted with chickens in which feed intake is limited to meet their maintenance energy requirements for a 6 day period, beginning 6 days post-hatch. Following realimentation the birds undergo compensatory growth that results in a greater proportion of lean versus adipose tissue at 8 wks of age compared to ad libitum fed birds. During energy restriction, hepatic enzyme activity associated with lipogenesis and in vitro lipogenesis were suppressed, followed by an overshoot at realimentation, and a subsequent suppression in activity during compensatory growth. The secretion of metabolic hormones were unaffected by nutrient restriction. Early post-hatch feed restriction was found to delay the proliferation of adipocytes but did not affect the development of normal adipocyte size. In summary, early feed restriction of short duration in chickens induces permanent changes in the mechanisms responsible for adipose tissue development.

24
RHIZOBIUM TRIFOLII POLYSACCHARIDES, OLIGOSACCHARIDES, AND OTHER METABOLITES AFFECTING DEVELOPMENT AND SYMBIOTIC INFECTION OF CLOVER ROOT HAIRS
Dazzo, Frank B., R. I. Hollingsworth, M. Abe, K. B. Smith, M. Welsch, P. J. Morris, S. Philip-Hollingsworth, & J. Salzwedel, Dept. of Microbiology, Michigan State Univ., East Lansing, MI 48824 USA

Rhizobium trifolii is a Gram negative bacterium which specifically infects root hairs of clover and induces nodule formation leading to a nitrogen-fixing symbiosis. We are interested in this infection process as a model of cell recognition between procaryotic and eucaryotic cells. Our studies show that certain surface polysaccharides of R. trifolii can modulate the number of infected clover root hairs. Oligosaccharide fragments derived from these polysaccharides retain this infection-related biological activity. In addition, R. trifolii excretes novel aromatic compounds which promote clover root hair development. Computer-interfaced image analysis using video microscopy shows that as little as 10 ng of one of the isolated aromatic compounds can increase root hair differentiation and extend growth. Further studies of the interaction between these microbial metabolites and the legume root should reveal important information on the root nodule symbiosis and help explain the impact of rhizosphere microorganisms on the normal growth and development of plant root systems in soil.

25
MYCORRHIZAE AND PLANT GROWTH AND DEVELOPMENT
Hadley, Geoffrey
Dept. of Plant Science, University of Aberdeen, Scotland, U.K.

Mycorrhizal fungi are known to be important in the enhancement of uptake of phosphate and nitrogen sources by many plants, and the uptake of carbon in early (post-germination) growth stages in orchids. Little is known about the specific mechanisms and interface transfer systems, however.

Apart from effects on nutrition, the assumption of hormonal relationships causing morphogenetic changes in host tissue has led to much speculation and interpretation of hypothetical models, illustrating developmental effects. Root growth and development in ectomycorrhizas is significantly changed by infection; VA mycorrhizal roots are not so markedly affected.

Developmental changes affecting stem anatomy and apical dominance can be recognised, together with some effects on meristems. Other characteristics such as specific leaf area, photosynthetic rates and carbon distribution are known to be influenced by infection in various mycorrhizal systems.

Progress in interpreting effects on plant growth and development is difficult. Applications in agriculture are less useful than formerly predicted but the ecological importance of mycorrhizae is wide-ranging.

26
AGROBACTERIUM AS A GENE VECTOR FOR PLANTS: TRANSFER OF GENES INVOLVED IN MORPHOGENESIS
Owens*, Lowell D. & Ann C. Smigocki, USDA-ARS, Tissue Culture and Molecular Biology Lab, Beltsville, MD 20705 USA

Tumor lines were derived from infection of soybean plants or excised cotyledons with Agrobacterium tumefaciens strains carrying Ti (tumor-inducing) plasmids mutated in the T-DNA (transferred-DNA) region. Tumor lines incited by strain tmr-338::Tn5 stably regenerated roots for more than a year. Lines incited by wild type strain A348 and mutant strains tms328::Tn5 and tml::Tn5 grew as unorganized callus. All tumor lines were characterized by elevated IAA levels compared to nontransformed soybean callus. The tml locus appeared to regulate auxin production by native auxin genes. Another T-DNA gene, the isopentenyl transferase gene, was cloned, reconstructed to provide altered levels of transcription, and inserted into a binary vector in A. tumefaciens strain LBA4404. Infections of several plant species with agrobacteria carrying these constructs produced tumors that displayed varying degrees of shoot induction depending on promoter strength.

27
THE EFFECTS OF INFECTION ON GROWTH
W. R. Beisel*, Dept. Immunology & Infectious Diseases, School of Hygiene & Public Health, The Johns Hopkins Univ., Baltimore, MD 21701 USA.

Generalized infectious diseases have long been known to cause losses of body weight and muscle mass of magnitudes that are roughly proportional to the severity of illness. These losses of body mass are accompanied, in infant or pre-adult hosts, by a cessation or faltering of growth. Depending on the severity and duration of illness, or in instances of closely occurring infections, "catch-up" growth in the post-illness period may be lacking or inadequate. Infections accompanied by fever are characterized by a hypermetabolic state. Metabolism during infection is further influenced by an increased output of ACTH, growth hormone, adrenocorticoids, insulin, and glucagon. The greatest catabolic effects, however, are induced by Interleukin-1 (IL-1), a hormone-like endogenous mediator produced by monocytes and other cells. IL-1 initiates fever, a catabolic breakdown of muscle protein, a broad reprograming of hepatocyte metabolism, shifts in trace element location, pancreatic secretion of insulin and glucagon, and the mobilization of neutrophiles. IL-1 also stimulates the immune system. While the many metabolic and physiologic effects of IL-1 are presumed to aid in survival of the host, they also have important costs. Losses of body nutrients and energy stores caused by IL-1 (and other hormones) result in the growth faltering of infection.

28
THE ROLE OF INTERLEUKIN-1 IN ENERGY BALANCE.
Kluger, Matthew J., Department of Physiology, The University of Michigan Medical School, Ann Arbor, MI 48109 USA

During infection, inflammation, or trauma many phagocytic cells release interleukin-1, a protein responsible for initiating dozens of physiologic, metabolic and immunologic actions. Many of these have profound effects on energy balance. Fever, particularly during the "chill phase," results in an increase in energy expenditure. The degradation of muscle protein and of bone, coupled with the synthesis of acute-phase proteins and the proliferation of lymphocytes, fibroblasts and other cells, would result in further increases in metabolism. The loss of food appetite would decrease energy input; the increase in lethargy (perhaps caused by headache and myalgia) and in sleep would decrease loss of energy. It is probable that these changes in energy blance represent a coordinated response of the organism to mobilize its host defense and immune responses. The concomitant decrease in appetite, coupled with headache, myalgia and increased sleep would decrease the amount of time spent foraging for food or engaging in other activity; this would have the added benefit of decreasing the likelihood of predation when the host is particularly vulnerable.

29
NUTRIENT - HORMONE INTERACTIONS DURING PARASITISM IN CATTLE
T. H. Elsasser, USDA, Agricultural Research Service, Ruminant Nutrition Laboratory, Beltsville, MD 20705.

Survival having the highest priority, animals will adjust their metabolism to insure that vital functions are adequately maintained during periods of nutritional or pathologic stress. Challenged with demands of reduced nutritional intake, parasitic load and the host responses to the infection, animals often suffer lasting consequences in terms of stunted growth and an inability to reach full growth potential. Experimental models of parasitism utilizing the organism Sarcocystis cruzi in conjunction with nutritional models have helped to explain the mechanism by which stunting occurs in animals. In general, effects of parasitic infection on growth were associated with changes in the endocrine axis beyond those directly attributable to nutritional stress alone. Specifically, changes were principally associated with alterations in the pituitary, pancreatic and hepatic axes. Especially noticable were prolonged reductions in circulating concentrations of insulin-like growth factor-1 and its plasma carrier protein and elevations in somatostatin. General hormonal trends suggested that, in young parasitized animals, metabolism is down regulated in terms of growth in order to conserve nutrients for survival and response to infection.

30
INFLUENCE OF PARASITISM ON GROWTH OF CATTLE MEDIATED THROUGH
TUMOR NECROSIS FACTOR
Fayer, Ronald*, USDA, ARS, Animal Parasitology Institute, Beltsville, MD 20705 USA

Poor growth, reduced feed efficiency, and a wasting diathesis characterized by atrophied skeletal muscles, hair loss and serous atrophy of fat mark chronic sarcocystosis in livestock. Despite a high prevalence of this protozoan parasite in cattle and sheep, the parasite burden per animal need not be great to elicit such signs and to stimulate a strong mononuclear cell reponse in many tissues. Detection of a parasite associated toxin that kills rabbits, induces gelation in the Limulus Amebocyte Assay, and stimulates production and release of tumor necrosis factor in murine macrophages suggests that the parasite exerts its effects through endogenous mediators.